

QUANTIFICATION OF LEWISITE IN AIR BY LIQUID CHROMATOGRAPHY MASS SPECTROMETRY (LC/MS)

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ABSTRACT

It has been determined that lewisite will hydrolyze and oxidize under ambient conditions to form chlorovinyl arsonic acid (CVRA) which is an As+5 compound. Most published methods for the analysis of lewisite require the derivatization of lewisite with a dithiol compound to produce a compound that can be analyzed using a gas chromatograph. The derivatization methods generally have poor reproducibility and precision which is due to the fact that the CVRA compound will not be derivatized by the dithiol compound. The authors have established an air monitoring method that will account for both lewisite and CVRA sampled from the air during various sampling periods. Both lewisite and CVRA are extracted off a solid sorbent tube and analyzed by liquid chromatography/mass spectrometry.

INTRODUCTION

Lewisite (2-chlorovinylchloroarsine) is a toxic arsenical compound. Since it is categorized as a suspected carcinogen, vapor concentrations must be monitored to protect worker safety in areas where the chemical is stored and where the compound is going to be neutralized. Several different air-monitoring methods have been developed and all have employed some sort of derivatization technique to quantify the amount of lewisite. The derivatization techniques rely on a chemical reaction of lewisite with dithiol to produce a compound that is more amenable to gas chromatography. These techniques have been plagued with poor precision and accuracy, particularly with aspiration times beyond two hours.

Research performed by the authors at the United States Army Chemical Agent Munitions Disposal System (USACAMDS) has culminated in a method with improved accuracy and precision compared to existing twelve hour methods. The method employs a glass solid sorbent tube packed with TenaxTA(60/40 mesh) that collects a vapor sample over a period of twelve hours at 0.5 liters per minute. Lewisite is collected on the sorbent tube, extracted with hexane and methanol and oxidized by adding 6% hydrogen peroxide. The extract is quantified by LC/MS.

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BACKGROUND

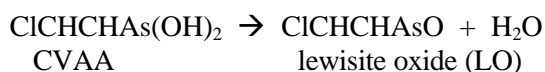
The chemistry of Lewisite will be discussed along with some of the experiments that were conducted to validate the reactions.

LEWISITE REACTIONS

Lewisite undergoes several different reactions. The most rapid is hydrolysis.



This reversible reaction can occur with a very small amount of water. CVAA can also lose a water molecule and form lewisite oxide (chlorovinylarsineoxide).



This reaction will only occur in the absence of water. The lewisite oxide will then polymerize. The polymerization reaction occurs slowly.

Lewisite is not easy to oxidize directly, but after it has hydrolyzed to CVAA oxidation occurs readily.



LEWISITE OXIDATION TO CVRA

The authors found that lewisite spiked on a sorbent tube that was aspirated over a period of time tended to have poor recovery (<30%). It was theorized that the poor recovery was attributed to the oxidation of CVAA. Humidity was hydrolyzing the lewisite to CVAA and the oxygen was oxidizing it to CVRA. To test this theory, stannous chloride pre-filters were placed at the sampling end of the tubes. Stannous chloride was used because of its ability to absorb the oxygen from the air. The samples were aspirated in the same manner as before and the lewisite recoveries were much improved (>90%).

LEWISITE OXIDATION PATHWAY AND RATE

To further test the theory and to find the reaction pathway and rate, two experiments were performed. The first was to determine if hydrolysis had to occur prior to oxidation. A set of glass impingers filled with water and another set filled with methanol were each spiked with 1 ug of lewisite. Compressed air was bubbled through the impingers at 0.5 liters per minute for one hour. The samples were analyzed for lewisite by HPLC/MSD. The impingers filled with methanol had a recovery of 90%. The impingers filled with water had a recovery of <10%. The results conclude that hydrolysis of the lewisite to CVAA is the first step in oxidation.

The second test was to estimate a reaction rate for the oxidation of Lewisite to CVRA. A set of sorbent tubes were spiked with 1080 ng of lewisite and aspirated for 5 minutes at 1

liter per minute. Another set of tubes were spiked with 1080 ng of lewisite and aspirated for 12 hours at 0.5 liters per minute. Each sample was analyzed for lewisite and CVRA. This process was repeated for 2 hours, 1 hour, 30 minutes, and 15 minutes. Based on the data, oxidation begins to occur between 5 minutes and 15 minutes of aspiration and then levels off after 15 minutes (see Figure 1). This is most likely due to the oxidation reaction reaching an equilibrium due to the concentration of oxygen in the air.

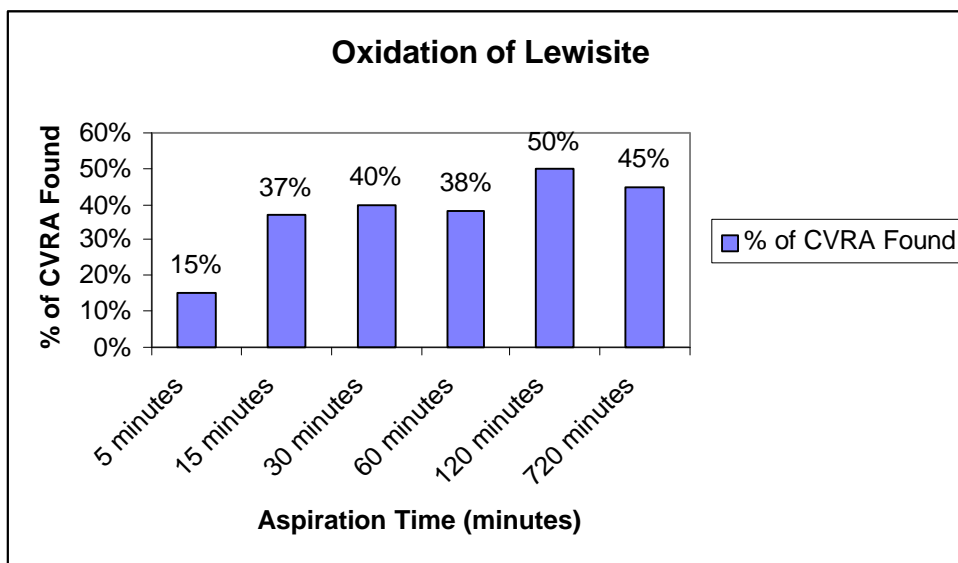


Figure 1. Oxidation of lewisite on a solid sorbent tube.

CVRA HEADSPACE TEST

Approximately 1 gram of felt pads were soaked in 697 ug/mL CVRA/methanol. The pads were placed in a heated chamber. The headspace was sampled with a sorbent tube for 1 hour. The sample was analyzed for CVRA by LC/MS. The entire procedure was performed at ambient temperature, 70 C and 150 C. There was < 0.1% CVRA found in the headspace, which draws a conclusion that CVRA is essentially not a vapor hazard.

The entire experiment was repeated using 897 ug/mL lewisite/methanol and a significant amount of lewisite was found on the tube. To compare the vapor pressure of CVRA with that of a known chemical agent, the experiment was again repeated using 425 ug/mL VX/isopropanol. The VX was analyzed by GC/FPD and a significant amount was recovered.

The authors believe that lewisite/CVAA will only oxidize on a substrate. So although CVRA is not a significant vapor hazard, it is a contact hazard and could be spread through the air by depositing on dust particles and being carried by air current.

METHODOLOGY

After reviewing the results of the previous experiments, specifically that lewisite will hydrolyze in air due to humidity and then oxidize as a function of time, the authors proposed a 12 hour air monitoring method that would work whether the lewisite was oxidized or not. Therefore a more accurate representation of the lewisite concentration in the sampled air would be obtained. The precision and accuracy study that was performed to validate the method is outlined below.

ASPIRATION

Twelve sorbent tubes were spiked in duplicate at 0, 216 ng, 540 ng, 864 ng, 1080 ng and 1620 ng. The spikes corresponded to 0.2, 0.5, 0.8, 1.0, 1.5 times the time weight average of 0.003 mg/m³. The tubes were aspirated for twelve hours at 0.5 liters per minute. This process was repeated for four days for a total of 48 samples.

EXTRACTION

Samples are extracted using a modified solid phase extraction system. The system consists of an extraction manifold, a vial rack and a vacuum pump. Plastic pipette tips are used to fit the sorbent tubes into the vacuum manifold (see Figure 2). The tip is inserted into the manifold and the sorbent tube is placed into the plastic tip. All extraction port valves are closed and the vacuum pump is switched on.

An aliquot 0.25 mL of hexane is applied to the top of the sorbent tube (see Figure 3) and slowly allowed to be pulled through the tube by opening the valve slightly. The tube is allowed to air dry for approximately 10 seconds after which the valve is closed. An additional 0.25 mL aliquot of hexane is applied to the top of the sorbent tube and allowed to flow through the sorbent in the same manner. The analyst moves on to the next tube in the rack.

After all tubes have been extracted with hexane, the analyst performs the same extraction procedure with 4 aliquots of methanol. Two different solvents are used because hexane extracts any lewisite present on the tube but will not extract CVRA and methanol extracts CVRA but will not adequately extract lewisite.

After the tubes have been extracted, 0.25 mL of 6% hydrogen peroxide is added to each vial. This oxidizes any lewisite or CVAA present into CVRA.



Figure 2. Solid sorbent tube extraction system.



Figure 3. Analyst adding solvent to sorbent tube during extraction procedure.

ANALYSIS

Samples are analyzed on an Agilent Technologies 1100 HPLC/MS. The mass spectrometer has a single quadrupole with atmospheric pressure ionization electrospray source (see Table 1 for list of set points). The sample analysis is performed in negative mode using

selective ion monitoring for ions 184.9 m/z, 186.9 m/z, 148.9 m/z and 122.9 m/z). The resulting data is identified by retention time and mass spectra ion ratios and quantified using the total ion chromatogram. Each set of 12 samples, are divided into two sets of 6, with each set being analyzed by a different analyst.

CALIBRATION

A standard solution of Lewisite at a concentration of 216 ug/mL Lewisite in methanol is used to calibrate the HPLC-MSD. Calibration curves are prepared by spiking solid sorbent tubes in duplicate with 216 ng, 1080 ng, and 1620ng of lewisite. After each injection of the standard solution, the sorbent tubes are aspirated for 5 minutes at approximately 1.0 liters/minute. The sorbent tubes are extracted and analyzed using the procedures defined above. The ChemStation software is used to record the calibration curve data and to quantify analyzed samples.

STATISTICAL RESULTS

All 48 data points were pooled into a single group and entered into a statistical analysis program called Certify. The program was used to plot the target concentration versus the found concentration for the data population. The average recovery of the samples was 92 %. The uncertainty in found mass was 38.04 %.

FIELD ACCEPTANCE

To test the long term stability of the new method a 30 day baseline study was performed. Every 12 hours a spiked tube (1080 ng Lewisite) and a blank tube were aspirated, extracted and analyzed following the procedures in the method. This process was repeated 5 days a week for 4 weeks. The agent recoveries are summarized in Figure 4.

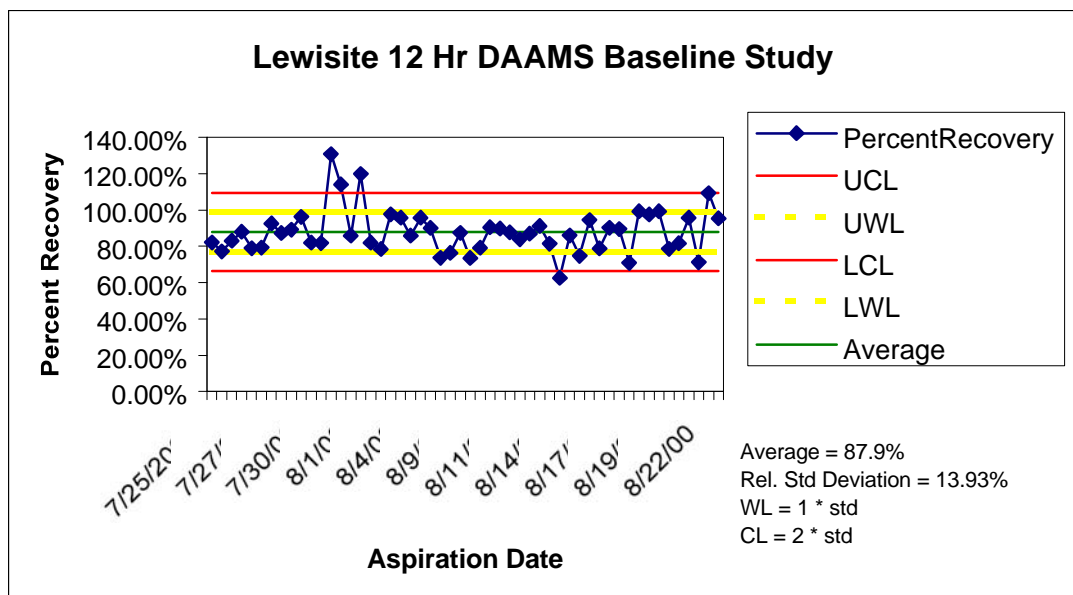


Figure 4. 12 Hour Baseline Summary.

INSTRUMENT CONFIGURATION

The following (Table 1) is a summary of the instrumentation used to develop the method and the key set points for the instrument.

TABLE 1. Instrument Set-points.

Instrumentation

Agilent Technologies 1100 High Performance Liquid Chromatograph with G1946B Mass Spectrometer using an Atmospheric Pressure Ionization Electrospray source.

Instrument Set Points for CVRA Method

Column: Agilent Technologies Extend C-18 2.1mm x 150 mm, 5 μ m

Pump Flow: 0.2 ml/min

Mobile Phase: 75/25 0.02 M NH₄OH/Methanol

Diode Array Detector: Not Used

MSD Ionization Mode: API-ES

Time Filter: Enabled

Peakwidth: 0.10 min

SIM Ion: 122.9 Fragmentor: 90

SIM Ion: 148.9 Fragmentor: 70

SIM Ion: 184.9 Fragmentor: 50

SIM Ion: 186.9 Fragmentor: 50

EMV Gain: 1.0

MSD Gas Temp: 350C

MSD Drying Gas: 12.0 L/min

MSD Nebulizer Pressure: 35 psig

MSD VCap: 4000 V

Injection Volume: 10 μ L

Column Thermostat: 25C

Expected Retention Time: 1.7 minutes

FUTURE ENDEAVORS

After reviewing each of the procedures in the method the greatest chance of induced error is during the extraction. It is a detail oriented task where analysts need to have good training and practice to produce precise and accurate results. Currently the issue is being addressed by developing an automated extraction system that would minimize the determinate error.

CONCLUSION

Derivatization methods are only reliable when Lewisite or CVAA is present. Most methods do not account for the loss of lewisite due to oxidation. If the lewisite is oxidized, the derivatization reaction will not work. The end result is low lewisite recoveries especially during longer aspiration times (>2 hours). Field-testing this method has demonstrated a viable new method of detection for lewisite with good precision and accuracy.

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